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Characterization of Amphiploid Hybrids between Bluebunch and Thickspike Wheatgrasses

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ABSTRACT

An amphiploid derivative from hybrids between bluebunch wheatgrass ($2n = 2x = 14$) [*Pseudoroegneria spicata* (Pursh) A. Love] and thickspike wheatgrass ($2n = 4x = 28$) [*Elymus lanceolatus* (Scribner and Smith) Gould] was developed to compare cytological, morphological, anatomical, and seed characteristics between the triploid F_1 hybrids ($2n = 3x = 21$) and their colchicine induced hexaploid ($2n = 6x = 42$) derivative (C_0). The amphiploid population produced an average of 3.1 seeds spikelet^{-1} and pollen stainability averaged 85.5%. Seed set under self-pollination in the amphiploids ranged from <1% to >99% with an average of 50%. Fifty-eight of the 103 plants studied were hexaploid ($2n = 6x = 42$). The remaining plants had chromosomes ranging from $2n = 39$ to $2n = 44$ and were less fertile than the euploids. Meiosis in the euploid plants was regular and typical of a segmental autoallohexaploid $St_1St_1St_2St_2HH$. The most common meiotic chromosome configuration was 21 bivalents. A high frequency of bivalents and a low frequency of quadrivalents indicate that the St genomes of the two parental species have diverged. The amphiploids had thicker culms, more leaves per culm, and longer and wider leaves than either parental species. Multivariate analysis demonstrated that the amphiploids, though morphologically similar to the parental species, was distinct. In general, C_0 hybrids were less vigorous than the F_1 from which they were derived. Through amphiploidy, genetic introgression between the two species is possible.

Bluebunch wheatgrass is one of the most important native bunch grasses in the Great Basin region and the Pacific Northwest. It inhabits dry mountain slopes at middle elevations with sagebrush and pinyon–juniper vegetation (Holmgren and Holmgren, 1977). Stands of bluebunch wheatgrass are often difficult to establish and, because of high palatability, become depleted under moderate to heavy grazing pressure, particularly at the boot stage (Miller et al., 1987). Bluebunch wheatgrass is comprised of diploid ($2n = 2x = 14$) and autotetraploid ($2n = 4x = 28$) forms containing the St genome (Stebbins and Snyder, 1956). The diploids are adapted to the more semiarid rangelands within the Great Basin while the tetraploids are restricted to the Pacific Northwest. Bluebunch wheatgrass is predominantly cross-pollinated (Jensen et al., 1990).

Thickspike wheatgrass is a cool-season perennial grass that is strongly rhizomatous and frequently glaucous (Holmgren and Holmgren, 1977). Because of the presence of rhizomes, thickspike wheatgrass is more persistent under repeated grazing than bluebunch wheatgrass. Thickspike is comprised of the St and H genomes, which associate as bivalents at metaphase I (Dewey, 1969). The St genome is derived from bluebunch wheatgrass and the H genome from *Hordeum* (Syn = *Critesion*; Dewey, 1984). Thickspike wheatgrass is largely cross-pollinated (Jensen et al., 1990). The goal was to create a hybrid between two native grasses that would combine the palatability of bluebunch wheatgrass with the persistence of thickspike wheatgrass.

On the basis of genomic structure of thickspike wheatgrass, Dewey (1965) hypothesized that it evolved through independent hybridization events involving either diploid bluebunch (StSt) and diploid *Hordeum* species (HH) or their autotetraploid counter parts (StStStSt; HHHH). Triploid F_1 hybrids between bluebunch and thickspike were meiotically irregular at metaphase I and failed to set seed under open-pollination (Dewey, 1965). However, in tetraploid hybrids (StStStH) between tetraploid bluebunch and thickspike, stainable pollen ranged from 0 to 50% and several of the hybrids produced 100 seeds plant^{-1} (Dewey, 1965). Chromosome pairing data in the triploid hybrid suggest that one of the two thickspike genomes was closely related with that of bluebunch wheatgrass (Dewey, 1965).

Although genetic introgression between the two species apparently occurs most frequently from the diploid to tetraploid level, rhizomatous forms of bluebunch occasionally occur on range sites where bluebunch and thickspike wheatgrasses grow sympatrically (Holmgren and Holmgren, 1977). They further concluded that these plants resulted from gene flow from thickspike to diploid bluebunch wheatgrass. Bowden (1965) suggested that Montana wheatgrass [*Agropyron ablicans* Scribner & Smith] originated from back crosses between thickspike × bluebunch hybrids with the thickspike parent. Wyoming wheatgrass [*A. griffithsii* Scribner and Smith], which is similar to Montana wheatgrass with the exception of its pubescent glumes and lemmas, apparently arose from crosses involving the pubescent form of thickspike. These hypotheses were supported by Dewey (1965, 1970) who obtained progeny and backcross derivatives from thickspike × bluebunch hybrids that closely resembled Montana and Wyoming wheatgrasses.

Crosses between species with different genomic compositions often result in sterile F_1 hybrids, which frequently leads to cytological instability and low fertility in newly formed amphiploids (Ashman and Boyle, 1955; Hill and Buckner, 1962). Dewey (1968) concluded that sterility, cytological irregularities, and lack of vegetative vigor in early amphiploid generations must be overcome if newly developed amphiploids are to have an impact in nature as new species or as newly developed cultivars. Within the wheatgrasses, breeders have overcome

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sterility barriers by doubling the chromosomes of sterile F_1 hybrids. Colchicine-induced tetraploids of diploid crested wheatgrass [*A. cristatum* (L.) Gaertn.] and diploid Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski] were used to introgress diploid and tetraploid germplasm (Asay et al., 1985, Jensen et al., 2005). The effect of chromosome doubling from diploid to autotetraploid often results in larger vegetative and floral parts (Eigsti and Dustin, 1955; Randolph, 1941; Stebbins, 1947). Induced tetraploids of Italian (*Lolium multiflorum* Lam.) and perennial ryegrasses (*L. perenne* L.) had greater seedling vigor than their diploid counterparts (Sjödin and Ellerström, 1986). Plants of diploid Russian wildrye tend to be shorter, finer stemmed, leafier, and generally higher in forage production than tetraploids (Asay et al., 1996). Tetraploids ($2n = 4x = 28$) have larger seeds and superior seedling vigor (Berdahl and Barker, 1991; Lawrence et al., 1990). Bingham et al. (1994) proposed that increased vigor of tetraploid alfalfa compared with diploid forms might be associated with greater complementary gene interaction at the tetraploid level. Tetraploid rye (*Secale cereale* L.) was 15% taller and had 12% fewer tillers than diploid rye (Muntzing, 1951). Kernel weight of rye increased 50% in the tetraploids (Muntzing, 1951). Guard cells and pollen grains are often significantly larger in polyploids than in their diploid progenitors. It has been speculated that polyploid species are more stable over a broader range of environments (Ellerston, 1959). Polyploid species of *Aegilops* are reported to be broadly adapted to a wider range of environments than are their diploid relatives (Zohary, 1965).

This paper cytologically and morphologically characterizes the bluebunch and thickspike amphiploid population and compares it to the triploid F_1 hybrid and parents. This population was previously released as a germplasm, SL-hybrid (Asay et al., 1991).

MATERIALS AND METHODS

Plant Materials and Populations

Plant nomenclature follows the "genomic system of classification" (Dewey, 1984) and genome designations are after Wang et al. (1995). Parental origin, hybrid development, chromosome pairing, and fertility of the parents, triploid, and tetraploid F_1 hybrids were reported by Dewey (1965). Populations representing thickspike, diploid bluebunch, the F_1 hybrid between bluebunch × thickspike, the original amphiploid (C_0), and the amphiploid breeding population were included in this study.

The original bluebunch (PI 232132) × thickspike (PI 236663) cross resulted in several triploid hybrids, from which the advanced generation breeding population was derived, designed to test the hypothesis that genetic introgression can occur between bluebunch and thickspike (Dewey, 1965). Both parents used in the initial cross were meiotically regular, forming predominately bivalents during metaphase I of meiosis (Dewey, 1965).

Colchicine-treated F_1 hybrids resulted in the production of relatively fertile hexaploid amphiploids, designated as the C_0 generation. The most fertile and vigorous plants from each succeeding generation were intercrossed to form the C_1 (first generation past chromosome doubling), C_2, and C_3 generations. Plants from the C_1, C_2, and C_3 generations were combined to form the initial amphiploid breeding population, which underwent four cycles of mass selection. During each cycle, approximately 65 plants were selected from a 3000-plant population on the basis of improved fertility, vegetative vigor, forage and seed yield, leafiness, and drought tolerance. The plants included in the present study were from the fourth breeding cycle.

Cytological Samples and Squash Preparations

Pollen mother cells from the hybrid populations were preserved in Carnoy's fixative (6:3:1 absolute alcohol–chloroform–glacial acetic acid) for 24 to 48 h, transferred to 70% ethanol, and stored in a refrigerator until analyzed. Squashed preparations of the pollen mother cells were stained with 2% acetocarmine. The frequency of univalents, bivalents, trivalents, and quadrivalents were determined in at least 10 microspore mother cells (616 total) from each of 50 euploid plants at metaphase I. Somatic chromosome numbers were determined at anaphase I based on a minimum of 10 cells from 100 amphiploid plants. The frequency of lagging chromosomes at anaphase I was determined in 50 cells from each of 50 plants. Micronuclei were counted in 100 postmeiotic microspore tetrads from each of 50 amphiploid plants.

Pollen Stainability and Seed Set

Spikes for pollen stainability were collected at anthesis from 100 amphiploid plants. The pollen grains were immersed in an I_2KI (iodine–potassium iodide) solution, which stains starch found in viable pollen grains black or dark gray. Aborted pollen grains are shrunken and light amber colored in I_2KI. A minimum of 1000 pollen grains were scored as viable or inviable in the amphiploid. Seed set under open-pollination for the amphiploid was determined on five spikes per plant 1 mo after anthesis. Self-fertility of the amphiploid was determined by isolating five spikes of the same 100 plants in parchment bags before anthesis. The spikes were hand threshed and seed counted to estimate plant fertility expressed as seeds spikelet^{-1}. Percentage self-fertility was computed by comparing the seed set spikelet^{-1} of the self-pollinated spikes with that of the open-pollinated spikes.

Morphological Traits

All plant materials were located at the Evans Research Farm approximately 2 km south of Logan, UT (41°45′ N, 111°8′ W, 1350 m above sea level). Soil at the site is a Nibley silty clay loam series (fine, mixed, active, mesic Aquic Argixerolls). The 40-yr (1951–1999) average annual precipitation at the site was 455 mm, with approximately one-half occurring from May through October.

Morphological variation in the parents and the advanced amphiploid population were measured on 18 morphological characters (Table 1). Forty-two mature plants each of bluebunch and thickspike were randomly selected from 10 and nine accessions, respectively, in a completely randomized design for inclusion in the morphological portion of the study. Bluebunch wheatgrass accessions included 'Whitmar', PI 236670, D-1252, D-2839, D-2840, D-2815, D-2837, D-2838, P-739, and a collection from Yellowjacket, ID. Accessions of thickspike wheatgrass included 'Critana' (Stroh et al., 1972), 'Elbee' (Smoliak and Johnston, 1985), 'Sodar' (Douglas and Ensign, 1954), D-2847, D-2848, D-2845, D-2850, PI 232115, and a collection from Wyoming. Accessions designated as D- were obtained from the Triticeae Collection at the Forage and Range Research Lab,

Table 1. Means for morphological characteristics for 42 plants each of bluebunch, thickspike, and the amphiploid breeding population.

Character	Population			LSD (0.05)
	Bluebunch	Thickspike	Amphiploid hybrid	
Culm length, cm	79.46 (52.8–98.0)	73.91 (55.1–90.5)	90.8 (71.2–112.6)	4.4
Leaf height on culm, cm	47.59 (28.2–70.0)	47.39 (24.2–66.6)	59.64 (46.9–74.4)	4.0
Leaf blade length, cm	18.09 (11.1–25.2)	14.0 (8.4–19.6)	22.1 (15.1–31.5)	1.5
Leaf blade width, mm	3.44 (2.2–5.3)	2.98 (2.3–3.9)	3.56 (2.6–4.6)	0.2
Leaf nodes per culm	3.4 (3–5)	3.6 (2–5)	4 (3–5)	0.2
Spikelets per spike	10.9 (7–17)	15.5 (11–22)	12.3 (8–16)	0.9
Florets per spikelet	8.6 (5–12)	6.1 (5–9)	6.7 (4–8)	0.4
Spike length, cm	16.26 (11.1–21.0)	12.9 (10.3–17.1)	13.79 (10.0–18.9)	0.9
Spikelet length, mm	20.62 (15.9–25.0)	14.62 (12.4–17.6)	19.44 (15.2–23.2)	0.9
First glume length, mm	8.26 (6.0–11.4)	6.59 (5.0–8.6)	8.63 (6.7–10.4)	0.5
First glume awn length, mm	0.18 (0–1.5)	0.0	0.93 (0.1–3.9)	0.2
First glume width, mm	1.7 (1.3–2.2)	12.7 (0.8–1.7)	1.7 (1.4–2.1)	0.1
Second glume length, mm	9.68 (6.5–12.3)	7.46 (5.7–9.2)	9.6 (7.6–11.4)	0.5
Second glume awn length, mm	0.24 (0–3.1)	0.0	1.08 (0.0–3.1)	0.3
Second glume width, mm	1.94 (1.5–2.6)	1.42 (0.9–1.9)	1.87 (1.5–2.5)	0.1
Lemma length, mm	10.79 (8.6–12.9)	9.2 (7.6–11.2)	11.83 (9.4–13.9)	0.5
Lemma awn length, mm	5.44 (0–18.6)	0.11 (0–1)	5.5 (0.4–13.1)	1.9
Lemma width, mm	2.74 (2.4–3.2)	2.46 (2.1–3.1)	3.02 (2.7–3.4)	0.1

USDA-ARS, Logan, UT. Morphological samples were taken at the onset of anthesis and measurements were made on five reproductive culms selected at random from each of 42 plants. The parental nursery was adjacent to the amphiploid breeding population from which 42 plants were randomly selected.

The effect of chromosome doubling was measured on 19 morphological characters (Table 2) in the triploid F_1 and C_0 (doubled F_1 hybrid) hybrids. Seven clones of one F_1 plant and eight C_0 clones, genetically identical to the F_1 except for ploidy level, were included in the study. Measurements were taken on seven reproductive culms per clone.

Impressions of the adaxial stomatal apparatus were obtained as described by Sinclair and Dunn (1961) and Hilu and Randall (1984). Clear nail polish was applied to the adaxial side of five blades plant^{-1} that had been previously soaked in water for 12 h. After 4 h, the nail polish was peeled off and mounted on a microscope. Length and width of 20 stomatal apparatus from each leaf (100 plant^{-1}) were measured with a micrometer at 400×.

Table 2. Mean characters for F_1 and C_0 amphiploid plants.

Character	Population means (range)	
	F_1 hybrids	C_0 amphiploid
Blade length, cm	13.5 (11.1–15.7)	16.7 (13.6–18.9)**
Blade width, mm	2.9 (2.5–3.2)	3.6 (3.3–3.7)**
Leaf nodes per culm	3.5 (3–4)	3.2 (3–4)**
Spike length, mm	11.1 (9.9–12.6)	12.6 (10.5–14.3)**
Spikelet length, mm	10.0 (14.4–16.9)	17.7 (16.4–19.8)**
Spikelets per spike	12.9 (10–15)	11.5 (11–12)**
Florets per spikelet	6.1 (5–8)	5.5 (5–7)**
First glume length, mm	6.4 (6.0–6.8)	8.9 (8.0–9.5)**
First glume awn length, mm	0.4 (0.3–0.5)	0.6 (0.3–0.8)**
First glume width, mm	1.5 (1.4–1.5)	1.7 (1.6–1.9)**
Second glume length, mm	7.2 (6.7–7.5)	9.9 (8.7–10.3)**
Second glume awn length, mm	0.3 (0.1–0.3)	0.5 (0.4–0.7)**
Second glume width, mm	1.6 (1.6–1.7)	1.9 (1.7–1.9)**
Lemma length, mm	9.5 (9.0–10.0)	11.8 (11.1–12.1)**
Lemma awn length, mm	0.5 (0.4–0.7)	0.8 (0.5–1.3)**
Lemma width, mm	2.5 (2.4–2.6)	2.9 (2.9–3.0)**
Culm width, mm	1.5 (1.3–1.6)	2.1 (1.7–2.0)**
Stomatal apparatus length, mm	0.032 (0.029–0.037)	0.040 (0.037–0.045)**
Stomatal apparatus width, mm	0.023 (0.020–0.027)	0.030 (0.026–0.037)**

** Significant at the 0.01 level in 1 d.f. (F_1 vs. C_0) contrasts.

Statistical Analysis

All data were subjected to ANOVA using GLM procedures as a fixed model with plants nested within populations. The MS associated with the variation among populations was tested for significance using the MS for variation among plants within populations as an error term. Mean separations were made on the basis of LSDs at the 0.05 probability level (SAS Institute, 1999). Correlation coefficients were computed based on entry × rep means using PROC CORR (SAS Institute, 1999). Principal components were derived using correlation matrices. Cluster analysis was performed using unweighted pair group mathematical average (UPGMA) algorithms on the distance matrices to provide a distance phenogram. The distance coefficient was defined as the average taxonomic distance computed by NT-SYS (Rohlf, 1992).

RESULTS AND DISCUSSION

Cytology

Chromosome pairing in bluebunch-2*x* and thickspike was regular, forming seven and 14 bivalents per cell, respectively, as reported by Dewey (1965). Chromosome pairing in the triploid hybrid (StStH) between bluebunch and thickspike averaged 6.84 univalents, 6.95 bivalents, and 0.08 trivalents per cell (Table 3) (Dewey, 1965). The high proportion of bivalents in the triploid hybrid (6–7 per cell) support the conclusion that there are close homologies between the St genomes in bluebunch and thickspike.

Chromosome numbers in the amphiploid breeding population ranged from $2n = 39$ to 42. Hyperploid plants accounted for 14% of the population and hypoploids made up 30%. Chromosome pairing in the amphiploid population was not consistent with what was expected based on the pairing associations in the triploid hybrid between bluebunch and thickspike. On the basis of the proposed genomic composition in the C_0 (StStStStHH) and the close association between the St genomes in the triploid hybrid (Table 3), chromosome associations with three or more chromosomes should be observed in most cells. The two most frequently observed chromosome pairing associations in the amphiploid breeding population

Table 3. Chromosome associations at metaphase I in hybrids between bluebunch and thickspike wheatgrasses.

Hybrids	Chromosome associations I	II	III	IV	V	No. of cells	% of total
Bluebunch 2*x* × thickspike	7	7	0	0	0	213	88.0
4*x* (triploid hybrids	6	6	1	0	0	14	5.9
StStH)	5	8	0	0	0	9	3.7
	9	6	0	0	0	2	0.8
	4	7	1	0	0	2	0.8
	5	5	2	0	0	2	0.8
Average	6.84	6.95	0.08	0	0		
Bluebunch 4*x*† × thickspike	7	3	5	0	0	12	6.2
4*x* (tetraploid hybrids	6	3	4	1	0	12	6.2
StStStH)	9	5	3	0	0	8	4.1
	6	5	4	0	0	8	4.1
	8	4	4	0	0	7	3.6
	8	7	2	0	0	7	3.6
	7	4	3	1	0	7	3.6
	8	1	6	0	0	6	3.1
	9	3	3	1	0	6	3.1
	5	5	3	1	0	6	3.1
	6	6	2	1	0	5	2.6
	6	4	2	2	0	5	2.6
	10	4	2	1	0	5	2.6
	8	5	2	1	0	5	2.6
	7	7	1	1	0	5	2.6
	7	6	3	0	0	4	2.0
	9	2	5	0	0	4	2.0
	6	8	2	0	0	4	2.0
	10	3	4	0	0	4	2.0
	9	8	1	0	0	4	2.0
	10	6	2	0	0	4	2.0
	8	2	4	1	0	4	2.0
Average	7.05	4.69	3.01	0.61	0		
Bluebunch 2*x* × thickspike	0	21	0	0	0	170	27.6
4*x* (amphiploid hybrids	0	19	0	1	0	140	22.7
StStStStHH)	2	20	0	0	0	73	11.9
	1	19	1	0	0	42	6.8
	2	18	0	1	0	41	6.7
	0	17	0	2	0	39	6.3
	1	17	1	1	0	29	4.7
	4	17	0	1	0	14	2.3
	2	16	0	2	0	13	2.1
	4	19	0	0	0	13	2.1
	3	18	1	0	0	9	1.5
	3	16	1	1	0	6	1.0
	0	15	0	3	0	5	0.8
	1	15	1	2	0	5	0.8
Average	0.95	19.03	0.17	0.63	0		

† Data taken from Dewey (1965).

were 21 bivalents and 19 bivalents and one quadrivalent (Fig. 1A; Table 3), which occurred in 28 and 23% of the cells, respectively. The occurrence of trivalents and quadrivalents in 55% of the metaphase I cells suggests the possible presence of a heterozygous interchange between the St genomes of bluebunch and thickspike. Unpaired chromosomes (Fig. 1B) were observed in 41% of the metaphase I cells and up to three quadrivalents cell^{-1} were observed in 47% of the cells (Fig. 1C). Chromosome paring in the amphiploid was more typical of a segmental autoallohexaploid with a genomic makeup of $St_1St_1St_2St_2HH$ than one with a genomic makeup of StStStStHH. Chromosome pairing in the amphiploid hybrid suggests that when the exact homologous chromosome is present, preferential pairing will occur. However, in the absence of an exact homologous chromosome (i.e., triploid hybrid), homeologous chromosomes are capable of pairing. Lagging chromosomes averaged 0.54 laggards cell^{-1}, which resulted in 0.70 micronuclei quartet^{-1}. There was a negative correlation between number of univalents and seeds spike^{-1} ($r = -0.29$, $P < 0.05$) and micronuclei and percentage stainable pollen ($r = -0.29$, $P < 0.05$) (data not shown). Univalent frequency was correlated with the frequency of lagging chromosomes ($r = 0.78$, $P < 0.01$) and the frequency of micronuclei quartet^{-1} ($r = 0.52$, $P < 0.01$). As the incidence of univalents cell^{-1} increased, percentage stainable pollen and seed set declined ($r = -0.29$, $P < 0.05$). Univalents were negatively correlated ($r = -0.52$, $P < 0.01$) with bivalent frequency.

Percentage Stainable Pollen and Seed Set

Percentage stainable pollen in the amphiploid breeding population ranged from 56 to 99% and averaged 85 ± 0.1% stainable pollen. This is similar to the 90% stainable pollen reported for bluebunch and thickspike (Dewey, 1965). Consistent with crested wheatgrass (Jensen et al., 2006), level of aneuploidy had no effect on percentage stainable pollen (data not shown). However, associated with aneuploidy in the amphiploid was a significant ($P < 0.05$) decrease in seeds spikelet^{-1} (data not shown). This similar effect was observed in the amphiploid hybrid between *E. lanceolatus* and *E. caninus* (Jensen, 2005) and in crested wheatgrass (Jensen et al., 2006).

Despite the parents being self-sterile, amphiploid plants were self-fertile (data not shown). Euploid plants produced 23% ($P < 0.05$) more seed under self-pollination than did aneuploid plants (data not shown). This may not be so surprising since most of the H genome *Hordeum* species are highly self-fertile (Jensen et al., 1990). In addition, autotetraploid forms of bluebunch have increased levels of self-fertility (Jensen et al., 1990). With the exception of the thickspike complex, all species

Fig. 1. Meiotic chromosome association for the bluebunch × thickspike amphiploid. (A) 19 II + 1 IV, (B) 1 I + 17 II + 1 III + IV, and (C) 15 II + 3 IV.

Table 4. Principle component analysis of morphological characters for 42 plants each of bluebunch, thickspike, and the amphiploid breeding population.

Character	Component 1	Component 2	Component 3
Culm length	0.707	−0.473	0.319
Leaf height on culm	0.529	−0.680	0.790
Leaf blade length	0.677	−0.097	−0.073
Leaf blade width	0.277	−0.708	0.415
Leaf nodes per culm	0.494	0.397	0.298
Spikelets per spike	0.839	0.380	0.012
Florets per spikelet	−0.478	−0.366	0.308
Spike length	0.502	0.644	0.077
Spikelet length	0.566	−0.008	0.055
First glume length	0.893	0.081	0.148
First glume awn length	0.465	−0.506	−0.606
First glume width	0.863	0.154	−0.065
Second glume length	0.876	0.177	0.193
Second glume awn length	0.437	−0.455	−0.690
Second glume width	0.838	0.193	0.025
Lemma length	0.876	−0.051	0.107
Lemma awn length	0.379	0.049	−0.700
Lemma width	0.822	−0.141	−0.103
% of total variation	44.9	14.63	11.32

comprised of the St and H genomes are capable of producing seed under self-pollination (Jensen et al., 1990).

Morphology

Principal component analysis was used to explore the multivariate patterns of variation among populations of bluebunch, thickspike, and the amphiploid breeding population. On the basis of 18 morphological characters (Table 1), principal components accounted for 71% of the variation in the first three axes and separated bluebunch, thickspike, and the amphiploid (Table 4, Fig. 2). Within the first component, which accounted for 45% of the variation, spikelets spike^{-1}, first and second glume length and width, lemma length and width all had factor loadings > 0.82. Component 2 was much less diagnostic, with spike length, leaf height on the culm, and leaf blade width having the highest weightings. Leaf height on the culm and glume and lemma awn lengths characterized principal component 3.

Bluebunch wheatgrass was characterized by having longer spikes ($P < 0.05$) and fewer spikelets spike^{-1} ($P < 0.05$) than thickspike and the amphiploid (Table 1). Characteristic of bluebunch are more distantly spaced spikelets on the rachis. Thickspike wheatgrass had more spikelets and shorter spikes than either the amphiploid or bluebunch populations. The amphiploid was intermediate to its parental species in both respects (Table 1). Culm length (cm) and leaf length (cm) and width (mm) in the amphiploid hybrid were significantly ($P < 0.05$) longer and wider than in bluebunch and thickspike. Leaves were oriented significantly ($P < 0.05$) higher on the culm with more leaf nodes per culm in the amphiploid hybrids than either parent.

Examination of the position of individual plants with respect to the first two principal component axes showed that the species tended to be located in different but contiguous portions of the cluster (Fig. 2). This was expected, since it has been hypothesized that bluebunch and thickspike have introgressed with each other over time. These observations were supported by Dewey (1965, 1970), who obtained progeny and backcross

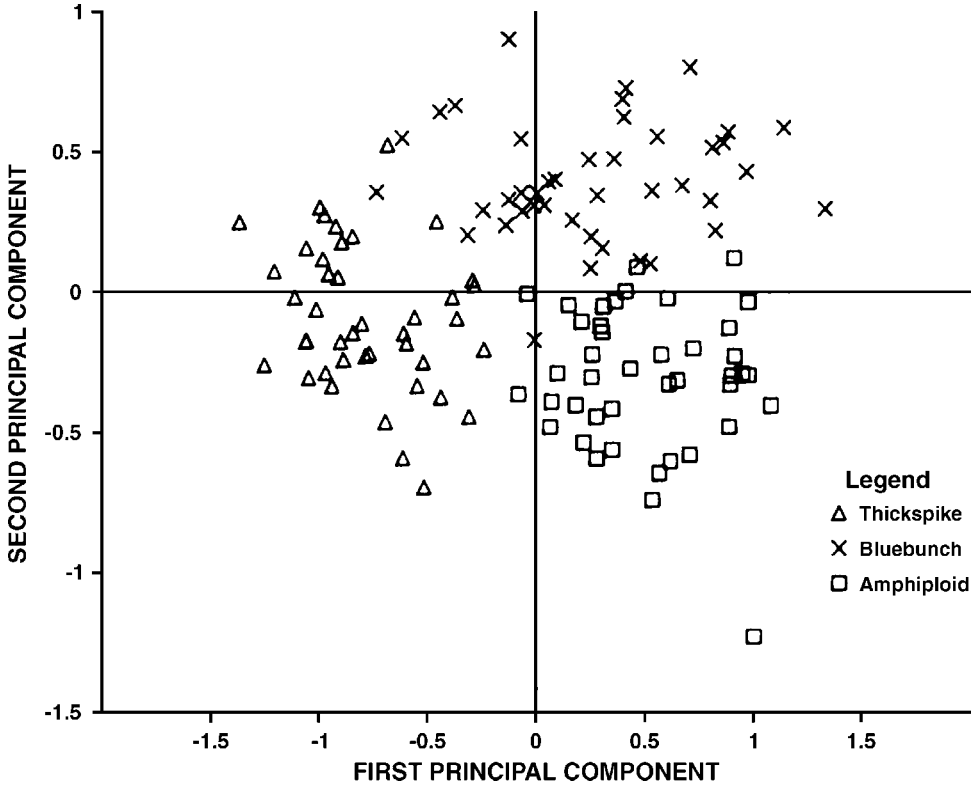

Fig. 2. Cluster analysis of 18 morphological traits for bluebunch, thickspike, and the amphiploid hybrid.

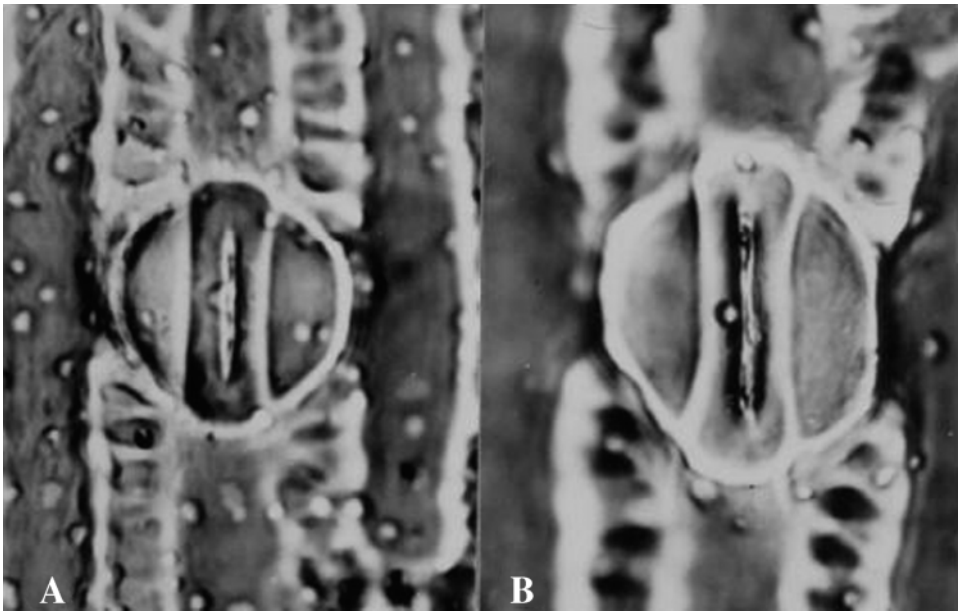

Fig. 3. Guard cells of the F_1 and C_0 hybrids showing the effect of chromosome doubling. (A) F_1 triploid hybrid and (B) C_0 amphiploid hybrid.

derivatives from thickspike × bluebunch hybrids that closely resembled Montana and Wyoming wheatgrasses.

Effect of Chromosome Doubling

All character contrasts between the F_1 hybrid and its genetically identical C_0 derivative were significant (Table 2). Compared with F_1 plants, C_0 plants had significantly ($P < 0.05$) longer and wider leaf blades, thicker culms, longer spikes and spikelets, longer and wider stomatal apparatus (Fig. 3), longer and wider first and second glumes, longer first and second glume awns, longer and wider lemmas and lemma awns (Table 2). The C_0 plants had fewer leaf nodes per culm and spikelets spike^{-1} (Table 2). The F_1 plants produced >2.5 times as many flowering culms as the C_0.

SUMMARY

The amphiploid breeding population derived from the hybrid bluebunch × thickspike was 56% euploid and 44% aneuploid. Meiosis in the euploids was essentially regular, 50% of the cells formed either 21 bivalents, or 19 bivalents and one quadrivalent. Aneuploid plants were significantly less fertile than their euploid counter parts. The frequency of aneuploidy can likely be reduced in subsequent generations by increasing the selection pressure for seed set (Jensen et al., 2005). The St genomes of bluebunch and thickspike are similar but display preferential pairing in the amphiploid. Self-pollination in the amphiploid breeding population ranged from highly self sterile to completely self fertile so that opportunities were evident for selection of either mode of pollination.

On the basis of the 18 floral and vegetative characteristics measured, the amphiploid hybrid was more similar to bluebunch than thickspike, but morphologically can be effectively separated from both parents. Studies of F_1 and C_0 counterparts indicated that induced amphiploidy in this population resulted in an initial reduction in vegetative vigor, larger guard cells and floral parts, and thicker stems. Through the use of chromosome doubling, this amphiploid successfully combined genes from of bluebunch and thickspike into a partially fertile hexaploid population that is vegetatively more vigorous than bluebunch wheatgrass. Reduced fertility and increased occurrence of aneuploid plants continue to be a problem in the amphiploid after four cycles of selection. However, there appears to be enough variation for pollen stainability and seed set to indicate that selection for increased fertility is possible. In addition, further selection emphasis on plants with a chromosome number of $2n = 6x = 42$ will likely increase fertility.

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